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ORIGINAL ARTICLE

Five-weekly S-1 plus cisplatin therapy combined with trastuzumab therapy in HER2-positive gastric cancer: a phase II trial and biomarker study (WJOG7212G)

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Abstract

Background Five-weekly S-1 plus cisplatin (SP) therapy is the standard care for advanced gastric or esophagogastric junction cancer (GC/EGJC) in East Asia. However, its efficacy and safety when combined with trastuzumab therapy for human epidermal growth factor receptor 2 (HER2)-positive advanced GC/EGJC remains unclear.

Methods Patients received 5-weekly SP therapy (S-1 at 40–60 mg twice daily for 21 days plus cisplatin at 60 mg/m^2 on day 8, every 5 weeks) plus trastuzumab therapy (first dose of 8 mg/kg, then 6 mg/kg every 3 weeks). The primary end point was the response rate, and the secondary end points included progression-free survival, overall survival, safety, and serum biomarker levels.

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Results Forty-four patients were enrolled. The response rate, progression-free survival, and overall survival were 61% (95% confidence interval 46–76%), 5.9 months, and 16.5 months respectively. The commonest grade 3 or grade 4 adverse events were neutropenia (30%) and anorexia (25%). A significantly higher response rate (92% vs 43%; $P = 0.008$) and longer progression-free survival (median 14.5 months vs 4.2 months; $P = 0.028$) were observed in patients with high ($n = 14$) compared with low ($n = 17$) pretreatment serum neuregulin 1 levels.
Conclusions Five-weekly SP therapy combined with trastuzumab therapy showed a good antitumor response and acceptable toxicity in HER2-positive advanced GC/EGJC. Serum neuregulin 1 might be associated with the efficacy of this treatment regimen.

Keywords Gastric adenocarcinoma · S-1 · Trastuzumab · Neuregulin 1 · First-line chemotherapy

Introduction

Trastuzumab is a humanized monoclonal antibody that inhibits human epidermal growth factor (EGF) receptor 2 (HER2) signaling and induces antibody-dependent cellular cytotoxicity [1]. Trastuzumab in combination with fluoropyrimidine plus cisplatin demonstrated a survival benefit in patients with HER2-positive advanced gastric or esophagogastric junction cancer (GC/EGJC) in the ToGA trial [2]. In the trial, most patients received a 3-weekly schedule of capecitabine and cisplatin (XP), which is a standard regimen for metastatic gastric cancer [2, 3]. In the Japanese subgroup of the ToGA trial, grade 3 and 4 adverse events were observed in 84% of patients in the trastuzumab arm [4]. Similar adverse events were also observed in another global trial, AVAGAST, in which XP was used as the backbone chemotherapy in combination with bevacizumab [4]. Thus, the standard dosage of the XP regimen appears to be high for a considerable proportion of patients. In Japan, the 5-weekly schedule of the oral fluoropyrimidine S-1 plus cisplatin (SP) has been the most popular first-line chemotherapy for advanced GC/EGJC since 2008, with an acceptable toxicity profile [5], and is widely used in East Asia. However, the efficacy and safety of 5-weekly SP therapy, in combination with trastuzumab therapy, has not yet been evaluated in HER2-positive advanced GC/EGJC.

Although basic research and clinical studies of breast cancer have investigated biomarkers associated with trastuzumab efficacy and resistance [6–10], there are few reports on biomarkers in patients with HER2-positive advanced GC/EGJC during trastuzumab treatment. Unfortunately, two anti-HER2 drugs—lapatinib (an EGF receptor and HER2 tyrosine kinase inhibitor) and trastuzumab emtansine—were unsuccessful in treating HER2-positive advanced GC/EGJC. These results suggest that there are biological differences between breast cancer and gastric cancer. Thus, to explore biomarkers relating to trastuzumab sensitivity or resistance in advanced GC/EGJC, we investigated baseline levels and sequential changes by following seven serum markers: HER2 extracellular domain, tissue inhibitor of metalloproteinase 1 (an inhibitor of HER2 shedding), EGF family members [neuregulin 1 (NRG1), EGF, and transforming growth factor α (TGF-α)], hepatocyte growth factor (HGF), and insulin-like growth factor 1 (IGF1).

We conducted a multi-institution single-arm phase II trial of 5-weekly SP therapy combined with trastuzumab therapy in HER2-positive advanced GC/EGJC to evaluate its efficacy and toxicity, and to explore circulating biomarkers related to the blockade of HER2 signaling with trastuzumab.

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Patients and methods

Patients

The main eligibility criteria were as follows: recurrent or unresectable GC/EGJC; HER2-positive tumors confirmed by immunohistochemistry scores of 3+ or 2+ and an HER2 to chromosome 17 ratio of 2.0 or greater by fluorescence in situ hybridization according to the routine procedure at each institution; age 20 years or older; Eastern Cooperative Oncology Group performance status 0–2; no history of chemotherapy except for adjuvant chemotherapy with fluoropyrimidine completed 6 months or more before enrolment; at least one measurable lesion as defined by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1; adequate oral intake; and preserved bone marrow and organ function. The study (WJOG7212) was performed by the West Japan Oncology Group, and was registered with the University Hospital Medical Information Network Clinical Trials Registry (protocol ID UMIN000008389).

Treatment schedule and assessment

Patients received SP in a 5-week cycle combined with trastuzumab in a 3-week cycle. S-1 was given orally twice daily for the first 21 days of each cycle, at a dose determined by body surface area (less than 1.25 m^2, 40 mg; 1.25–1.5 m^2, 50 mg; more than 1.5 m^2, 60 mg). Cisplatin at 60 mg/m^2 was given intravenously on day 8 of each 5-week cycle, for up to a total of eight cycles. S-1 and trastuzumab were given after discontinuation of cisplatin therapy. Trastuzumab was given intravenously at a loading dose of 8 mg/kg, and then at a dose of 6 mg/kg every 3 weeks. Adverse events were graded with use of Common Terminology Criteria for Adverse Events version 4.0. The S-1 and/or cisplatin dose was reduced if patients experienced any of the following adverse events during the preceding cycle: febrile neutropenia, neutrophil counts less than 500/mm^3, platelet counts less than 25,000/mm^3, creatinine clearance less than 50 ml/min when the serum creatinine level was 1.2–1.5 mg/dl, serum creatinine level greater than 1.5 mg/dl, grade 2 or grade 3 peripheral sensory neuropathy, grade 3 or grade 4 diarrhea, oral mucositis, anorexia, nausea, or vomiting. Trastuzumab alone was allowed as a protocol treatment if SP therapy was discontinued. Trastuzumab therapy was suspended if patients had symptomatic heart failure or a left ventricular ejection fraction less than 50%. During the suspension of trastuzumab therapy, S-1 and/or cisplatin administration was allowed. The study treatment was discontinued if the disease progressed, unacceptably severe toxicity occurred, or the patient requested discontinuation. Radiologic tumor evaluation was performed every 6 weeks according to RECIST version 1.1.

Serum sample collection and analysis

We collected serum samples at four time points: before treatment (baseline), immediately before the second and fourth trastuzumab administrations, and after confirmation of progressive disease (PD). Serum HER2 levels were measured by a chemiluminescence immunoassay (Siemens Healthcare Diagnostic, Tokyo, Japan). All other serum markers were measured by enzyme-linked immunosorbent assay (ELISA): EGF, TGF-α, and NRG1 levels were measured with DuoSet ELISA Development Systems kits (R&D Systems, Minneapolis, MN, USA), and HGF, IGF1, and tissue inhibitor of metalloproteinase 1 were measured with Quantikine human immunoassay kits (R&D Systems). All assays were performed in duplicate. The lower limits of detection were 3.91 pg/ml for EGF, 7.81 pg/ml for TGF-α, and 62.5 pg/ml for NRG1.

Statistical analysis

The full analysis set (FAS) was defined as all enrolled patients, excluding those who were judged ineligible for this study after registration. The per-protocol set (PPS) was defined as all patients in the FAS excluding patients whose efficacy could not be evaluated for any reason, and patients who had major protocol deviations or violations with respect to dosage, dose schedule, and prohibited combination therapies. The safety analysis set (SAS) was defined as all patients who received at least one dose of the study medication.

The primary end point was the response rate (RR) in the FAS, as evaluated by an independent review committee (IRC) composed of one medical oncologist and one radiologist. The RR in the PPS was assessed as a reference. Secondary end points included progression-free survival (PFS) in the FAS evaluated by the IRC, overall survival (OS) in the FAS, and safety in the SAS. PFS was defined as the time from the date of enrolment to the date of death from any cause, or to the date when PD was confirmed by radiologic imaging. Patients without PD were censored on the last confirmed date of non-PD. Patients who underwent curative resection were censored on the date of surgery. OS was defined as the time from the date of enrolment to the date of death from any cause. The investigators' evaluations of response and progression were adopted as the reference for clinical efficacy in this trial. The associations between circulating biomarkers and

clinical outcomes were investigated with use of the RR and PFS as determined by the IRC.

For a power of 80% with a one-sided alpha of 10%, 35 patients were initially required to reject an RR of 40% or less, with an expected RR of 60%. During the study, the protocol was amended on the basis of the favorable accrual rate. To improve precision, patient enrolment continued until 55 patients were enrolled (power 85%; one-sided alpha of 5%) or until the end of the predetermined accrual period, whichever came first.

Serum biomarker levels at the four collection points were compared with paired *t* tests. For each collection point, biomarker levels were compared between responders (complete response plus partial response) and nonresponders (stable disease, PD, and not evaluable) with the Mann–Whitney *U* test. PFS and OS were evaluated with the Kaplan–Meier method, and were compared between high and low marker groups (cutoff was the median serum level at the baseline) by a log-rank test. The RR was also compared by χ^2 tests. For all analyses, $p < 0.05$ was considered significant. No multiplicity adjustments were applied in the biomarker analyses because of the exploratory nature of this study.

Results

Patient characteristics

Between August 2012 and January 2014, 44 patients were enrolled across 21 hospitals in Japan. All patients were included in the FAS and SAS. Four patients were excluded from the PPS because they had measurable lesions not identified by the IRC. Patient and disease characteristics are shown in Table 1. The median age was 64.5 years (range 31–77 years). The performance status was 0 or 1 in 42 patients (95.5%). The primary tumor sites were the stomach in 37 patients (84.1%) and the esophagogastric junction in seven patients (15.9%). Sixteen patients (36.4%) had histologically poorly differentiated adenocarcinoma. Most patients (72.7%) had HER2 immunohistochemistry score 3+ tumors.

Table 1 Patient characteristics in the full analysis set ($n = 44$)

	Value
Age (years)	
Median	64.5
Range	31–77
Sex	
Male	34 (77.3%)
Female	10 (22.7%)
Performance status	
0	30 (68.2%)
1	12 (27.3%)
2	2 (4.5%)
Primary tumor site	
Stomach	37 (84.1%)
Esophagogastric junction	7 (15.9%)
Number of metastatic sites	
0–1	24 (54.5%)
≥2	20 (45.5%)
Metastatic sites	
Liver	19 (43.2%)
Lung	7 (15.9%)
Distant lymph nodes	24 (54.5%)
Peritoneum	14 (31.8%)
Bone	2 (4.5%)
Histologic type	
Papillary adenocarcinoma	3 (6.8%)
Tubular adenocarcinoma	20 (45.5%)
Poorly differentiated	16 (36.4%)
Signet-ring cell	2 (4.5%)
Prior therapy	
Gastrectomy[a]	6 (13.6%)
Adjuvant chemotherapy	1 (2.3%)
Unresectable/recurrent disease[b]	
Unresectable	43 (97.7%)
Recurrent	1 (2.3%)
HER2 status	
IHC score 2+, FISH positive	12 (27.3%)
IHC score 3+	32 (72.7%)

FISH fluorescence in situ hybridization, *IHC* immunohistochemistry

[a] Four patients underwent total gastrectomy and two patients underwent distal gastrectomy

[b] One patient had disease recurrence more than 6 months after prior gastrectomy and adjuvant chemotherapy

Efficacy

The cutoff date for analyses was December 2014, with a median follow-up time of 19.3 months (range 4.3–24.7 months). Forty-four patients in the FAS provided a statistical power of 81%, with a one-sided alpha of 5%. The RR judged by the IRC was 61.4% [95% confidence interval (CI), 45.5–75.6%; one-sided $P = 0.001$]. Two patients (4.5%) achieved complete response (Table 2). The median PFS evaluated by the IRC was 5.9 months (95% CI 5.0–9.3 months; Fig. 1a) and the median OS was 16.5 months (95% CI 14.3–21.6 months; Fig. 1b). In the PPS, the RR judged by the IRC was 67.5% (95% CI 50.9–81.4%) and the median PFS evaluated by the IRC was 5.9 months (95% CI 5.0–9.3 months). The RRs and median PFSs judged by the investigators and the IRC were similar (Table 2).

Safety

Table 3 shows the frequency of adverse events of all grades, and of grades 3 and 4. The grade 3 or grade 4 adverse events (10% or more of patients) were neutropenia (29.5%), anorexia (25.0%), anemia (18.2%), fatigue (13.6%), nausea (11.4%), diarrhea (11.4%), thrombocytopenia (11.4%), and hypoalbuminemia (11.4%). Palmar–plantar erythrodysesthesia syndrome was observed in 18.2% of patients (all less than grade 3). Febrile neutropenia occurred in two patients (4.5%). One sudden death occurred during the study treatment and was judged to be treatment related, although the precise cause of death was not identified.

Treatment exposure and postprotocol treatment

The median number of treatment cycles of S-1, cisplatin, and trastuzumab were 5.0 (range 1.0–17.0 cycles), 5.0 (range 0–8.0 cycles), and 8.5 (range 1.0–29.0 cycles) respectively. Five patients were still receiving the protocol treatment at the cutoff date for analysis. The remaining 39 patients discontinued the protocol treatment because of disease progression ($n = 27$, 61.4%), patient requests related to adverse events [$n = 4$ (anorexia, $n = 1$; fatigue, $n = 1$; unknown, $n = 2$), 9.1%], surgery after conversion to a resectable status ($n = 3$, 6.8%), grade 4 sepsis ($n = 1$, 2.3%), death during protocol treatment ($n = 1$, 2.3%), patient request for personal reasons ($n = 1$, 2.3%), achievement of complete response ($n = 1$, 2.3%), and the investigator's decision because of toxicity ($n = 1$, 2.3%). During the protocol treatment, no patient discontinued trastuzumab therapy. Three patients discontinued SP therapy, and afterward all of them continued trastuzumab therapy alone as the protocol treatment. One patient discontinued cisplatin therapy alone, and then continued with S-1 and trastuzumab therapy as the protocol treatment. Detailed information regarding drug therapy discontinuation during protocol treatment is shown in Table S1. Thirty-two patients (72.7%) received subsequent chemotherapy after discontinuing the protocol treatment (Table S2). Taxane-based regimens were most frequently used as second-line chemotherapy. Of note, approximately 40% of patients received a trastuzumab-containing regimen ($n = 16$) or trastuzumab emtansine ($n = 3$) beyond disease progression.

Serum biomarker assessment

Serum samples were available for 31 of the 44 patients (70.5%). The characteristics of this subgroup were similar to those of the entire cohort (Table S3). The numbers of samples collected at each time point were 31 before

Table 2 Tumor response

	FAS ($n = 44$)		PPS ($n = 40$)	
	IRC	Investigators	IRC	Investigators
Complete response	2 (4.5%)	2 (4.5%)	2 (5.0%)	2 (5.0%)
Partial response	25 (56.8%)	23 (52.3%)	25 (62.5%)	22 (55.0%)
Stable disease	9 (20.5%)	13 (29.5%)	9 (22.5%)	11 (27.5%)
Progressive disease	4 (9.1%)	4 (9.1%)	4 (10.0%)	4 (10.0%)
Not evaluable	4 (9.1%)	2 (4.5%)	0	1 (2.5%)
Objective response	27 (61.4%; 95% CI 45.5–75.6%)	25 (56.8%; 95% CI 42.2–70.3)	27 (67.5%; 95% CI 50.9–81.4)	24 (60.0%; 95% CI 44.6–73.7)
Disease control	36 (81.8%)	38 (86.4%)	36 (90.0%)	35 (87.5%)

CI confidence interval, *FAS* full analysis set, *IRC* independent review committee, *PPS* per-protocol set

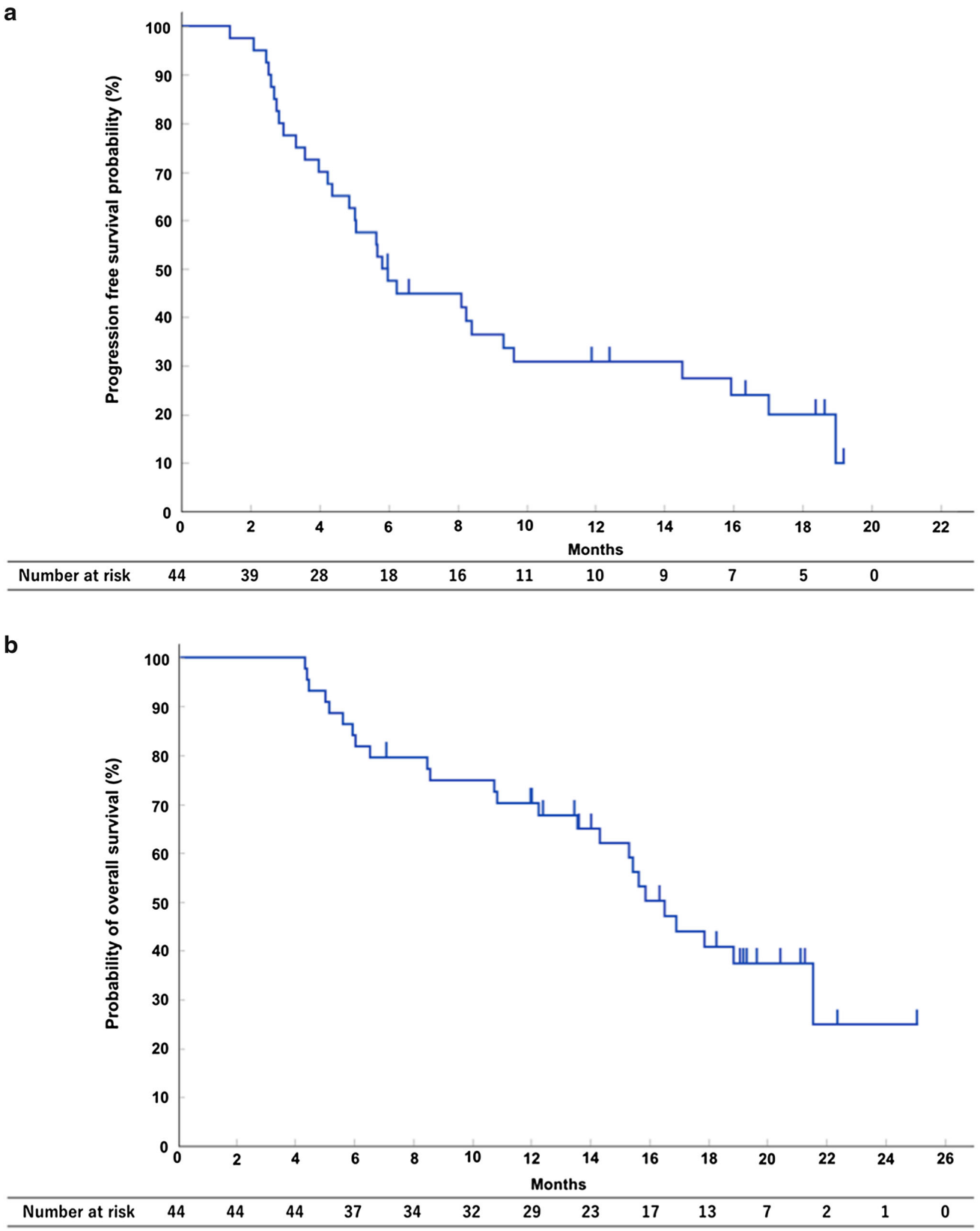


Fig. 1 **a** Progression-free survival evaluated by an independent review committee and **b** overall survival in the full analysis set

Table 3 Adverse events ($n = 44$)

	All grades	Grades 3 and 4
Hematologic adverse events		
Neutropenia	29 (65.9%)	13 (29.5%)
Anemia	38 (86.4%)	8 (18.2%)
Thrombocytopenia	16 (36.4%)	5 (11.4%)
Febrile neutropenia	2 (4.5%)	2 (4.5%)
Gastrointestinal adverse events		
Nausea	27 (61.4%)	5 (11.4%)
Vomiting	11 (25.0%)	1 (2.3%)
Diarrhea	22 (50.0%)	5 (11.4%)
Stomatitis	20 (45.5%)	2 (4.5%)
Other adverse events		
Anorexia	36 (81.8%)	11 (25.0%)
Fatigue	33 (75.0%)	6 (13.6%)
Palmar–plantar erythrodysesthesia syndrome	8 (18.2%)	0
Maculopapular rash	6 (13.6%)	0
Pyrexia	14 (31.8%)	0
AST level increase	21 (47.7%)	3 (6.8%)
ALT level increase	16 (36.4%)	4 (9.1%)
Total bilirubin level increase	9 (20.5%)	0
Creatinine level increase	11 (25.0%)	0
Hypoalbuminemia	26 (59.1%)	5 (11.4%)

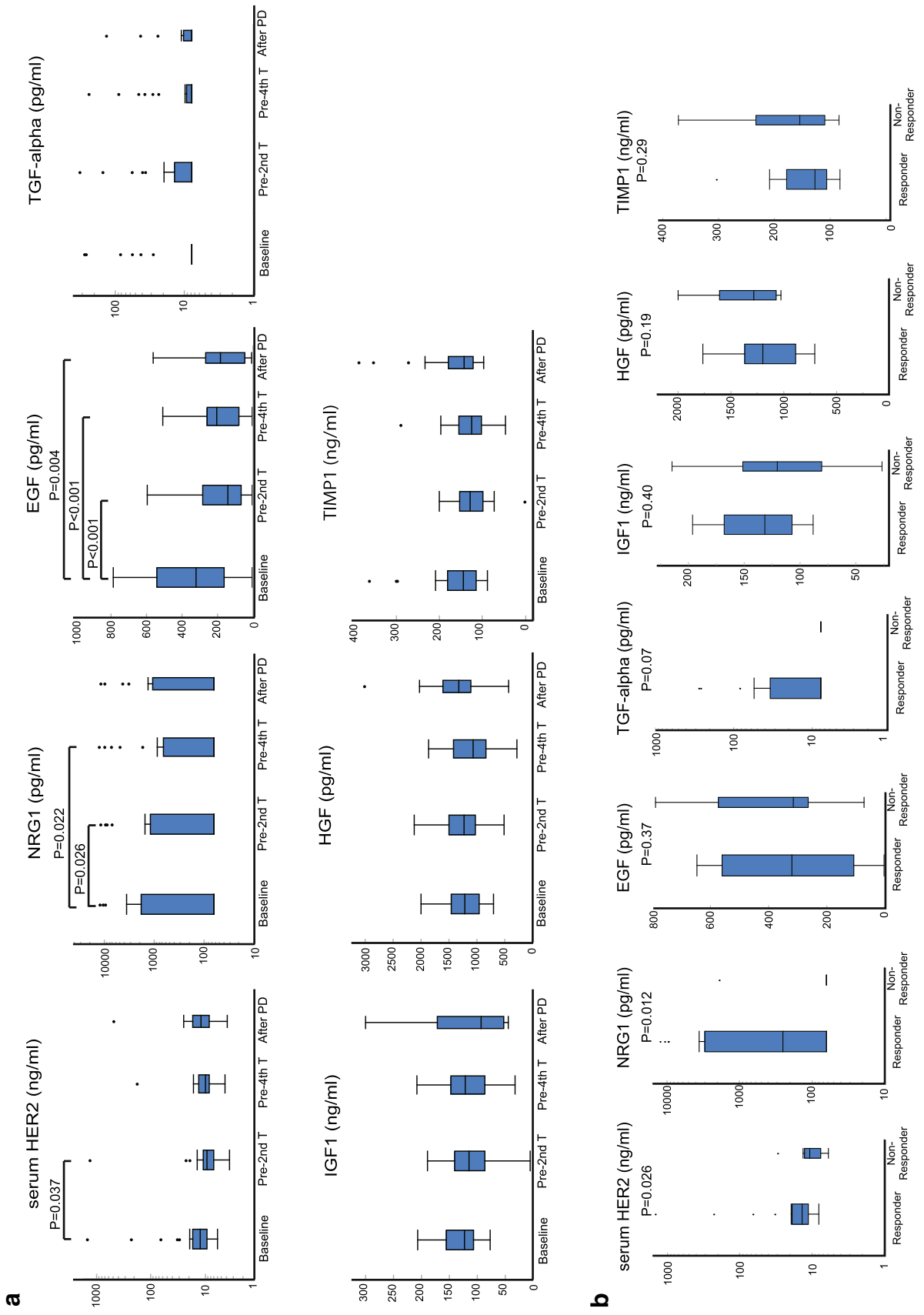
ALT alanine transaminase, *AST* aspartate transaminase

treatment as a baseline, 31 before the second trastuzumab administration, 28 before the fourth trastuzumab administration, and 18 after PD confirmation. HER2, NRG1, and EGF levels decreased after treatment ($P < 0.05$), but the levels of the remaining biomarkers showed no significant change from the baseline (Fig. 2a). None of the biomarker levels increased significantly after PD. Baseline levels of HER2 and NRG1 were significantly higher in responders ($n = 21$) than in nonresponders ($n = 10$; mean ± standard error for HER2, 96 ± 68 ng/ml vs 12 ± 2 ng/ml, $P = 0.026$; for NRG1, 2490 ± 883 pg/ml vs 242 ± 180 pg/ml, $P = 0.012$; Fig. 2b). The high and low marker groups were defined in the study protocol as being above and below the median serum level at the baseline respectively. However, because more than 50% of patients had baseline NRG1 levels below the lower detection limit (62.5 pg/ml), we divided the patients into high (detectable) and low (undetectable) NRG1 groups on the basis of that cutoff. The high baseline NRG1 group ($n = 14$) had a higher RR (92% vs 43%, $P = 0.008$) and longer PFS (median 14.5 months vs 4.2 months, $P = 0.028$) than the low baseline NRG1 group ($n = 17$; Fig. 3a). However, there was no significant difference in OS between the two groups (median not reached vs 13.6 months, $P = 0.053$; Fig. 3b). Similarly, the high baseline HER2 group (higher than the median value, 12.5 ng/ml, at the baseline) had a significantly higher RR (87% vs 50%, $P = 0.025$) but, in this case, there were no differences in either PFS or OS between the high and low baseline HER2 groups (Fig. S1). Although we analyzed the associations between clinical outcomes and changes in serum biomarker levels from the baseline during the study treatment, no significant associations were detected (Table S4).

Discussion

The present study demonstrated that 5-weekly SP therapy combined with trastuzumab therapy has promising antitumor activity and acceptable toxicity as the first-line chemotherapy for patients with HER2-positive advanced GC/EGJC. The primary end point of the RR was met, and we found that serum NRG1 levels may be a useful predictive biomarker for trastuzumab efficacy in this disease.

Analysis of the Japanese subpopulation in the ToGA study demonstrated that the RR, median PFS, and median OS of the patients in the trastuzumab plus XP arm were 64.4%, 6.2 months, and 15.9 months respectively [13]. Patient characteristics differed slightly between our study and the Japanese subgroup of the ToGA trial. All patients in our study had high HER2 expression, which was true of only 68.6% of the Japanese subgroup in the XP plus


a
serum HER2 (ng/ml)
P=0.037
NRG1 (pg/ml)
P=0.022
P=0.026
EGF (pg/ml)
P=0.004
P<0.001
P<0.001
TGF-alpha (pg/ml)
IGF1 (ng/ml)
HGF (pg/ml)
TIMP1 (ng/ml)
Baseline
Pre-2nd T
Pre-4th T
After PD
b
serum HER2 (ng/ml)
P=0.026
NRG1 (pg/ml)
P=0.012
EGF (pg/ml)
P=0.37
TGF-alpha (pg/ml)
P=0.07
IGF1 (ng/ml)
P=0.40
HGF (pg/ml)
P=0.19
TIMP1 (ng/ml)
P=0.29
Responder
Non-Responder

◀**Fig. 2** **a** Serum biomarker levels before treatment (baseline), before the second trastuzumab administration (*pre-2nd T*), before the fourth trastuzumab administration (*pre-4th T*), and after diagnosis of progressive disease (*PD*). Statistical differences between collection points were assessed by paired *t* tests. **b** Serum biomarker levels at the baseline in the responder and nonresponder groups. Statistical differences between the groups were calculated by the Mann–Whitney *U* test. Responder, patients with complete or partial responses; nonresponder, patients with stable disease, PD, or not evaluable. *EGF* epidermal growth factor, *HER2* human epidermal growth factor receptor 2, *HGF* hepatocyte growth factor, *IGF1* insulin-like growth factor 1, *NRG1* neuregulin 1, *TGF* transforming growth factor, *TIMP1* tissue inhibitor of metalloproteinase 1

trastuzumab arm of the ToGA trial. In addition, 40.9% of our patients had diffuse-type advanced GC/EGJC, compared with 9.8% in the ToGA trial. Patients with high HER2 expression are reported to be likelier to respond to trastuzumab plus XP treatment, and, conversely, trastuzumab plus XP treatment is less effective in patients with histologically diffuse-type tumors [2]. Although a cross-trial comparison is difficult, the efficacy results from our study and the ToGA trial appear to be comparable.

The results of another phase II trial of SP therapy combined with trastuzumab therapy, based on a 3-weekly schedule, have been published [14]. Compared with our study, the 3-weekly treatment regimen resulted in a longer median PFS (7.8 months) but a similar RR (68%) and OS (16 months). In the SOS study comparing 3-weekly and 5-weekly SP therapy, the 3-weekly SP regimen resulted in a longer PFS, similar OS, and a slightly higher toxicity than the 5-weekly SP regimen [15]. Taken together, these results suggest that although PFS may be slightly prolonged with 3-weekly SP threapy plus trastuzumab therapy, 5-weekly SP therapy can be considered to be a comparable combination chemotherapy with trastuzumab in HER2-positive advanced GC/EGJC.

OS was a secondary end point, and thus the follow-up period was relatively short in this study; yet, despite this limitation, OS appeared to be long. Most patients received subsequent chemotherapy: approximately half were treated with HER2-targeted regimens in our trial, although little information on HER2-targeting therapy beyond progression was available. This may have influenced the long OS in this trial. To study this issue, our group is conducting a randomized phase II trial (WJOG7112G) evaluating the efficacy of trastuzumab therapy continuation beyond progression (UMIN000009297).

The toxicity profiles of 5-weekly SP therapy combined with trastuzumab therapy were comparable to those of XP or 3-weekly SP therapy plus trastuzumab therapy; however, there were some differences in the frequency and severity of the adverse events. Palmar–plantar erythrodysesthesia syndrome was less common in our trial (18.2%, all grades) than in an XP plus trastuzumab therapy study (41.0%) [13]. Similarly, we observed increased creatinine level less frequently (25.0%, all grades) than in a 3-weekly SP therapy plus trastuzumab therapy study (45.0%) [14]. Anemia and neutropenia were commoner and severer in the 3-weekly SP therapy arm of the SOS study than in our study. Moreover, treatment discontinuation because of adverse events occurred less frequently in our study (15.9%) than in the 3-weekly SP therapy plus trastuzumab therapy study (31%). These findings support the proposition that 5-weekly SP therapy plus trastuzumab therapy is a feasible regimen.

In terms of a biomarker for trastuzumab, HER2 extracellular domain shed into the circulation from tumor cells has been reported to be a good biomarker for probing HER2 expression and monitoring its dynamic change, as it reflected patients' responses to HER2-targeted therapy in breast cancer [11]. However, its reliability as a biomarker remains controversial [6, 12]. As a mechanism of trastuzumab resistance, EGF families (NRG1, EGF, and TGF-α), HGF, and IGF1 signal pathways can activate downstream signaling of HER2 [8–10]. In this study, we investigated the expression levels of these markers during treatment. We identified a significant association between high serum NRG1 levels and better efficacy (RR and PFS). As an EGF-like ligand, NRG1 is a polypeptide growth factor that binds to human epidermal growth factor receptor 3 (HER3) and human epidermal growth factor receptor 4 [16, 17]. NRG1 has been considered to be a negative biomarker for trastuzumab-containing therapy as it activates heterodimerization of HER2 and HER3. Furthermore, overexpression of NRG1 in gastric cancer was reported to be associated with tumor progression by its regulating the self-renewal of cancer stem cells [18]. However, patients with breast cancer who overexpress transmembrane NRG1 have been shown to benefit from trastuzumab-based therapies [19]. Intriguingly, trastuzumab can inhibit NRG1-induced HER2 and HER3 heterodimerization [20]. Our results suggest that trastuzumab inhibits tumor progression via effects on NRG1, and this might contribute, at least in part, to its clinical efficacy in HER2-positive advanced GC/EGJC.

Circulating HER2 was expected to be a predictive marker for HER2 expression in gastric cancer tissues. Several studies have shown a significant correlation between serum HER2 levels and tissue HER2 expression [21–23]. However, in our study, serum HER2 levels ranged from undetectable to extremely high, even though our cohort was limited to patients with high-HER2-expressing tumors. Our study also demonstrated that high serum HER2 levels were associated with better RR but not PFS or OS. These data do not support the predictability of serum HER2 levels for trastuzumab efficacy.

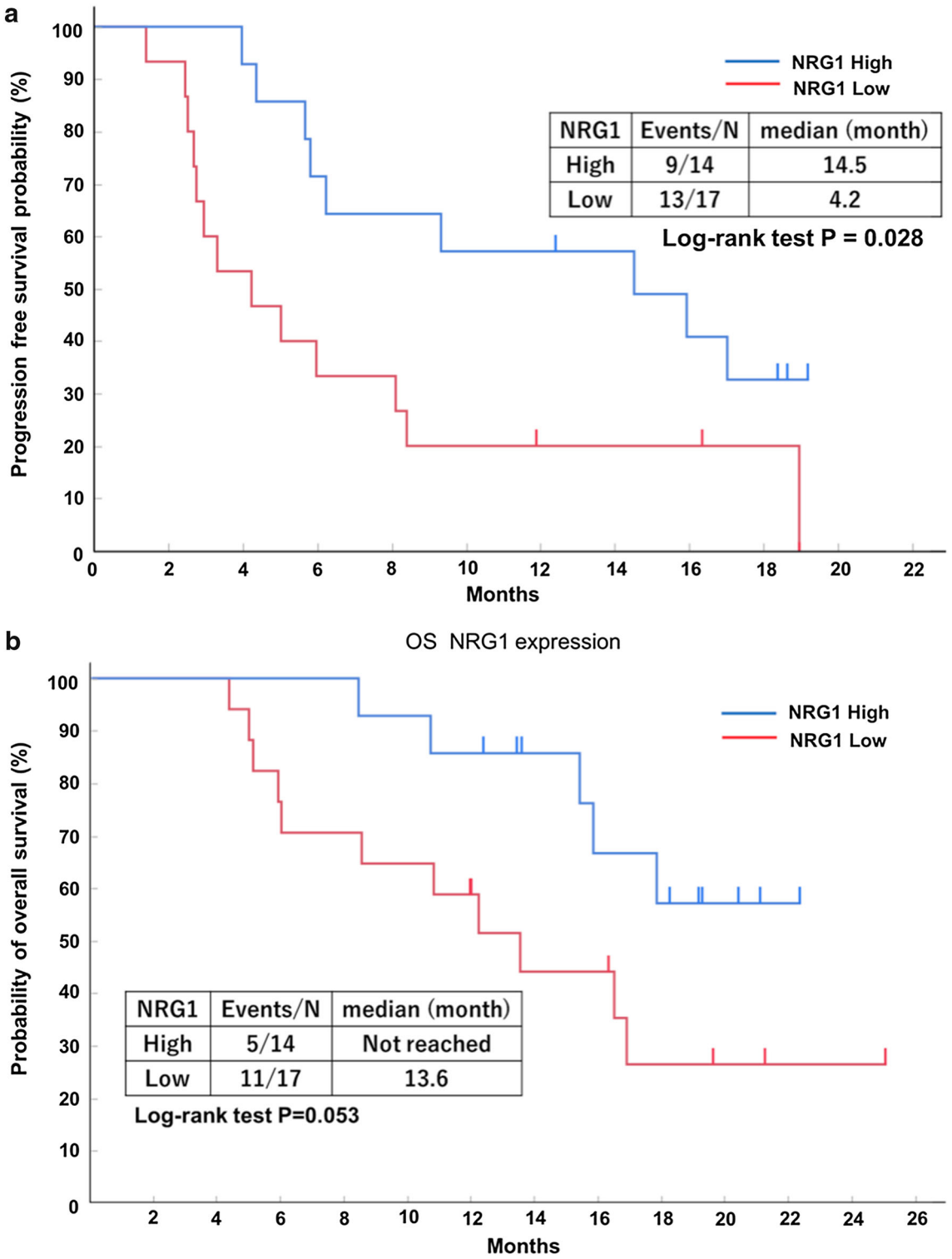


Fig. 3 **a** Progression-free survival and **b** overall survival (*OS*) of patients stratified by serum levels of neuregulin 1 (*NRG1*)

Changes in circulating HER2 levels during chemotherapy were reported to be associated with clinical outcomes in HER2-positive gastric cancer patients receiving trastuzumab and chemotherapy [21, 22]. Although we observed a decrease in serum HER2 levels in our study, such decreases simply appeared to reflect tumor shrinkage. It is likely that the decreases in EGF and NRG1 levels seen after chemotherapy could be accounted for by the same explanation. With regard to resistance to trastuzumab therapy, we found no significant change in serum biomarker levels

at disease progression. Thus, the clinical significance of monitoring these biomarkers must await further investigation.

Our study has some limitations. First, this was a single-arm phase II study with a small sample size, and therefore included some bias. Second, serum biomarker samples were collected from only 70% of the patients enrolled in the study, and the statistical power was insufficient to analyze multiple markers. Third, no standardized method was used to evaluate serum markers, including optimized cutoff levels.

In conclusion, 5-weekly SP therapy plus trastuzumab therapy showed good antitumor effects with acceptable toxicity for patients with HER2-positive advanced GC/EGJC. Further studies are needed to probe whether serum NRG1 is a candidate biomarker of this regimen.

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Compliance with ethical standards

Conflict of interest Yuji Miura received honoraria from Novartis and Kyowa Hakko Kirin. Ichinosuke Hyodo received honoraria from Taiho, Chugai, Daiichi-Sankyo, Yakult-Honsha, and Eli Lilly. Toshikazu Moriwaki received honoraria from Taiho, Chugai, and Takeda and research funding from Taiho, Sanofi, Boehringer Ingelheim, and MSD. Kazuhiro Nishikawa received honoraria from Taiho, Chugai, Yakult, and Ajinomoto. Naotoshi Sugimoto received research funding from Taiho, Daiichi-Sankyo, and Eli Lilly. Kenichi Yoshimura received honoraria from Taiho and Chugai. The remaining authors declare that they have no conflict of interest.

Human rights statement and informed consent All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. Informed consent (biomarker sampling was not mandatory) or a substitute for it was obtained from all patients included in the study, and the ethics committee of each institution approved all study procedures.

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